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Rapid report

Mutations in X-linked ichthyosis disrupt the active site structure of estrone/DHEA sulfatase

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Abstract

X-linked ichthyosis is an inherited genetic disorder of the skin that results from steroid sulfatase (STS) deficiency. Seven critical point mutations have been previously reported for the STS gene, six leading to amino acid substitutions and one to a premature termination of the polypeptide chain. The three-dimensional structure of the full-length human enzyme has been recently determined. Amino acid substitutions due to point mutations in X-linked ichthyosis are mapped onto the three-dimensional structure of human STS. In each case, the substitution appears to cause disruption of the active site architecture or to interfere with the enzyme's putative membrane-associating motifs crucial to the integrity of the catalytic cleft, thereby providing an explanation for the loss of STS activity.

Keywords: Estrone/DHEA sulfatase; Steroid sulfatase; Ichthyosis; Mutation; Three-dimensional structure; Molecular basis of steroid sulfatse deficiency

X-linked ichthyosis is an inherited genetic disorder of the skin that results from estrone (E1)/dehydroepiandrosterone (DHEA) sulfatase (ES) or steroid sulfatase (STS) deficiency [2,8]. While in the majority of these cases, there are extensive deletions of the gene, several point mutations, each corresponding to a single base change, have been identified among patients with the disease [1–3,8,12,15]. STS is found in several tissues, including human placenta, skin fibroblasts, breasts and fallopian tubes [4–6,9,11, 13,14,16,17]. The membrane-bound enzyme is distributed in the rough endoplasmic reticulum (ER) including the perinuclear cisternae, the Golgi cisternae, endocytic structures and the plasma membrane [11]. Localization of STS in the smooth and rough ER was demonstrated by immunohistochemical labeling [9]. The enzyme, along with cytochrome P450 aromatase and 17β-hydroxysteroid dehydrogenase 1 (17HSD1), is responsible for maintaining high levels of the active estrogen, 17β-estradiol (E2), in breast tumor cells. STS catalyzes the hydrolysis of E1-sulfate, which is subsequently reduced to E2 by 17HSD1. Intracrine biosynthesis by P450arom, STS and 17HSD1 in the breast accounts for most of the estrogens in post-menopausal women and, hence, could be a major factor in hormonal breast cancers [10].

The three-dimensional structure of the full-length STS from human placenta has recently been determined by X-ray crystallography [6,7]. The tertiary structure, shown in Fig. 1a, consists of two domains—a globular (55×60×70 Å), polar domain containing the catalytic site, and the putative transmembrane domain consisting of two antiparallel hydrophobic helices α8 and α9. The polar domain consists of two sub-domains with the α/β sandwich fold. Sub-domain 1 (SD1: N-terminal sub-domain), wound around a central 11-stranded mixed β-sheet flanked by 13 α-helices/helical turns, contains the catalytic core. Sub-domain 2 (SD2), consisting of roughly 110 C-terminal residues and wound around a four-stranded antiparallel β-beta sheet (strands 13–16) flanked by α helix 16, packs against turn and loop regions of the beta sheet of SD1. The two putative

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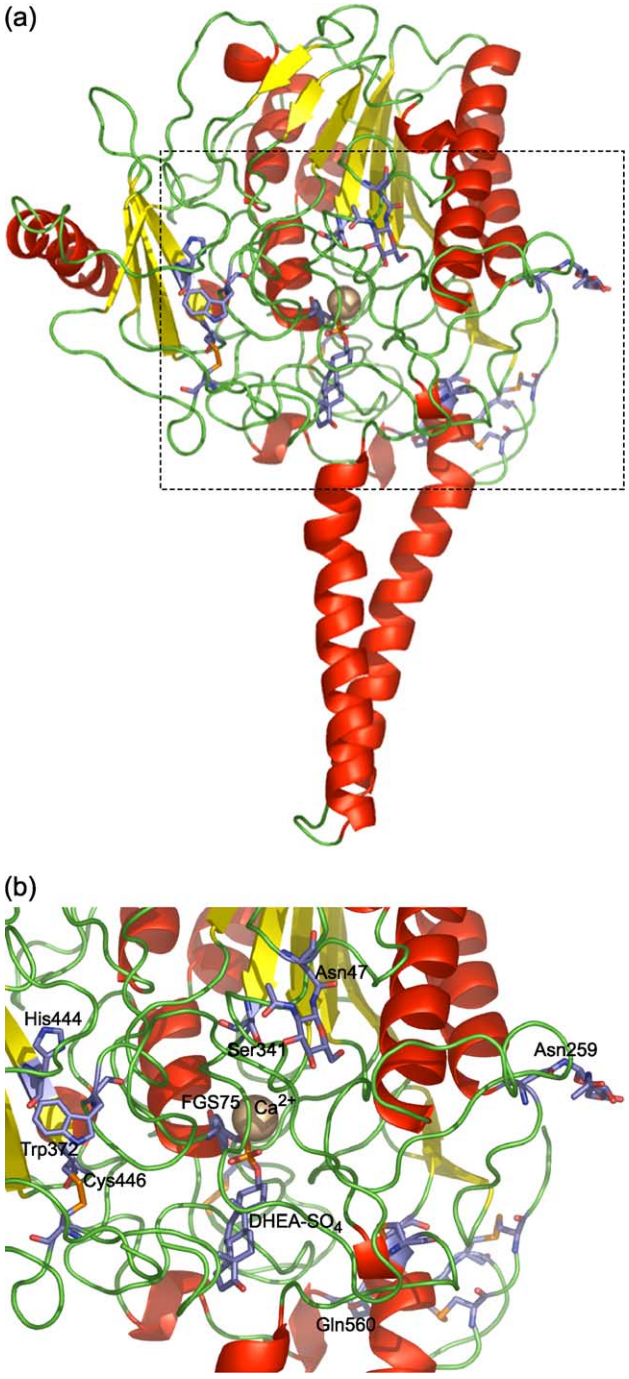

Fig. 1. (a) A ribbon diagram showing the secondary and tertiary structures of estrone/DHEA sulfatase. Sheets are drawn in yellow, helices in red and loop regions in green. Side chain atoms are color-coded (carbon, light blue; nitrogen, dark blue; oxygen, red; sulfur, orange). Backbone atoms are also displayed with their side chains. A magnified view of the boxed region is shown in panel (b). Point mutation sites display amino acids of the wild-type enzyme (Ser341, Trp372, His444, Cys446 and Gln560). The catalytic residue FGS75, the bivalent cation Ca^{2+} and a modeled DHEA-sulfate are shown at the active site. Two observed glycosylation sites (Asn47 and Asn259), each with one molecule of *N*-acetyl glucosamine, are also shown.

transmembrane helices protrude on one side of the nearly spherical polar domain, thereby giving the overall molecule a "mushroom-like" shape.

The location of the transmembrane domain is such that the opening to the active site, buried deep in a cavity in the "gill" of the "mushroom", rests near the membrane surface. A close-up view of the catalytic site is shown in Fig. 1b. The catalytic amino acid hydroxylformylglycine (FG) 75, created by posttranslational modification of Cys75, was found to be covalently linked to a sulfate moiety (as a sulfate ester of FG, denoted by FGS) [7]. The bivalent cation required for the STS activity was interpreted to be a Ca^{2+} [7]. The oxygen atoms of the Asp35, Asp36, Asp342, Gln343 and FGS75 side chains serve as ligands to the bivalent cation. In addition to the transmembrane domain, structural evidences suggest that several extended loop regions, especially two polypeptide segments, Phe548 to Leu568 that is spatially proximal to the active site groove, and Phe468 to Pro500 that consists of several hydrophobic side chains and display high thermal motion, associate with the lipid bilayer [7].

Seven critical point mutations have been previously reported for the STS gene, six leading to amino acid substitutions and one to a premature termination of the polypeptide chain [1,8,12]. Six amino acid substitutions are Ser341Leu, Trp372Arg, Trp372Pro, His444Arg, Cys446Tyr and Gln560Pro. A seventh mutation, a 19-base pair insertion at the exon 8–intron 8 splice junction at nucleotide 1477, results in a shift in the open reading frame and termination of the chain at residue 427, eight residues after the frameshift. Each of these mutations results in complete loss of STS activity [1] and hence the body's ability to synthesize active steroid hormones from sulfate conjugates. In Fig. 1a and b, all of the above wild-type amino acid side chains, the catalytic residue FGS75 and the bivalent cation Ca^{2+} are displayed. A DHEA-sulfate molecule, modeled in the active site by superimposing its sulfate moiety with the observed position of the sulfate group of FGS75, mimics an intermediate step of the catalytic reaction. In addition, two glycosylated asparagine side chains, Asn47 and Asn259, each linked to one experimentally observed *N*-acetyl glucosamine molecule, are also shown.

Ser341 is situated at the end of β9 on a sharp turn before Asp342 and Gln343 which contributes two oxygen ligands to Ca^{2+}. Furthermore, the side chain hydroxyl group of Ser341 is at a hydrogen bond forming distance (2.9 Å) from the backbone amide of Leu37, adjacent to other two ligands Asp35 and Asp36 at the end of β1, within a tight pocket. A Ser to Leu substitution here would cause complete disruption of the packing of backbones of both strands and hence the active site architecture. Our modeling result suggests that a Leu side chain here cannot be accommodated without causing serious steric conflicts. In contrast, Trp372 belongs to a long loop region with high thermal motion, about 16 Å away from the active site Ca^{2+}, preceding the one-turn helix α13. Although no direct disruption of the tertiary structure or the active site could be perceived by either Trp372Arg or Trp372Pro substitution, this side chain

packs against Phe467 and Phe503 side chains and is adjacent to a β-turn that contributes the Lys368 side chain, a catalytically important amino acid [7], to the active site. The backbone conformation of Trp372 could possibly allow for a proline at the position. The partially exposed nature of the loop could also accommodate a charged arginine side chain with some difficulty. However, it is conceivable that either of these substitutions interferes with the precise orientation of the Lys368 side chain, conserved among all three human arylsulfatases [7].

The next three substitutions are in the C-terminal sub-domain, which exhibits higher overall thermal motion than the N-terminal sub-domain. His444 is the last residue on the β-strand 13, roughly 17 Å away from the catalytic Ca^{2+} and buried in a somewhat hydrophobic pocket on the second β-sheet. Two charged residues in the pocket are Arg454 and Lys465 from neighboring strands; however, both are neutralized by approaching negatively charged Glu373 and Glu511 side chains, respectively. An arginine substitution here not only causes charge imbalance but also introduces steric clashes within the hydrophobic pocket, destabilizing the tertiary structure of the entire sub-domain that flanks the active site cleft. Two residues upstream along the strand is the reside Cys446, disulfide bridged to Cys489 and adjacent to another disulfide bridge between Cys487 and Cys481. The last three cysteine residues belong to a long loop described above that presumably associates with the lipid bilayer and is adjacent to the active site groove. Although a tyrosine side chain could be accommodated at position 446, breakage of the disulfide bond would interfere with the integrity of the β-sheet and the tertiary structure of the loop, thereby destabilizing the active site structure. All of the disulfide bridges in this part of the molecule appear to be critically positioned to maintain integrity of the loop conformations at or near the protein–lipid interface.

The Gln560 side chain is 22 Å away from the Ca^{2+} ion, on the opposite side of the active site cleft, shown in Fig. 1b, at the end of a helical turn (α18: residues 556–559). Interestingly, however, it belongs to the second loop that possibly associates with membrane, as previously described. Furthermore, side chain carboxyl group of Gln560 makes a strong hydrogen bond by accepting the amide proton from the Trp555 aromatic ring. Trp555, along with Trp550 and Trp558, are three important aromatic side chains that are thought to line the lipid interface and hence crucial to the function of STS as an ER membrane-bound enzyme [7]. While Gln560 is also present in human arylsulfatase A, none of these three tryptophan residues is present in arylsulfatases A or B, two water-soluble enzymes without the transmembrane domain. Although the backbone conformation of Gln560 in STS permits a proline substitution here, the loss of the Gln560 to Trp555 hydrogen bond may alter the orientation of the Trp555 side chain, which in turn may change the interactions of these residues at the polar face of the bilayer with lipid carbonyl groups. The presence of these aromatic side chains and their precise orientations at the polar lipid interface could be important not only for the membrane association of the enzyme but also for the passage of a large sulfated hydrophobic moiety, such as DHEA-sulfate, to the active site. Finally, the premature termination of the polypeptide chain at residue 427, situated at the beginning of the C-terminal sub-domain, completely wipes out the sub-domain and hence a section of the catalytic cavity, along with the membrane-anchoring loops. Such a mutation, if it yields a translatable mRNA, will result in an inactive enzyme.

Mapping of point mutations onto the three-dimensional structure thus suggests that known point mutations in STS deficiency cause severe destabilization of the active site architecture, thereby probably inactivating the enzyme. It is conceivable that some of the mutations could lead to unstable transcriptional products or interfere with the formation of folding intermediates resulting in unfolded proteins.

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